Title: Vessel differentiation in isolated stem segments of Fraxinus excelsior L. after treatment with auxin

Author: Ewa U. Kurczyńska

ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 61, nr 3–4: 343-357
1992

Vessel differentiation in isolated stem segments of *Fraxinus excelsior* L. after treatment with auxin

EWA U. KURCZYŃSKA

Department of Biophysics and Cell Biology, Silesian University,
ul. Jagiellońska 28, 40-032 Katowice, Poland

(Received: February 5, 1991. Accepted: December 30, 1991)

Abstract

Experiments were carried out on isolated stem segments of ash collected from natural conditions during the winter dormancy season. In order to stimulate cambial activity and vessel differentiation the internodes were treated with auxin. The duration of treatment with auxin was different in the particular experimental variants. It appeared that auxin was necessary not only for induction of vessel differentiation but also during their maturation. The results are discussed on the basis of S a c h s' (1981) hypothesis.

Key words: *auxin*, *cambium*, *vessel differentiation*, *Fraxinus excelsior*

INTRODUCTION

The fact that auxin is a necessary factor for vessel differentiation has been known for a long time (J a c o b s 1952). We also know that we can distinguish at least two stages in the process of vessel differentiation: determination and maturation (R o b e r t s 1976). The question arises whether auxin is necessary during both of these processes. From surgical experiments carried out on bean epicotyls we know that auxin is necessary during the entire time of redifferentiation of parenchyma cells into vessels and its polar flow is most important in this process (S a c h s 1974). Furthermore, from callus experiments we know that differentiation of tracheary elements depends on auxin concentration (W e t m o r e and S o r o k i n 1955).

The purpose of this work was to determine the required duration of auxin action and to answer the question whether it is the auxin concentration or its flow that is more important in the process of vessel differentiation in trees.

MATERIAL AND METHODS

Two year old stems of *Fraxinus excelsior* L. collected from natural conditions in January were used in the experiments. In laboratory the stems were cut into 12 cm long segments comprising one single internode without buds.

The water culture method was used, in darkness, at room temperature, maintaining 100% relative air humidity. The experiment was started in the first half of January, lasted 11 days and was repeated twice (in the second half of January and in the first half of February). The experiment included 6 variants described in "Results".

In each variant, internodes were divided into 6 groups on the basis of the length of auxin treatment. Each group contained 8 internodes (internodes used in the experiments were two years old and without any signs of cambial activity; the cambial zone comprised 3 to 4 of the fusiform cells in the radial file with thick radial walls of these cells). Auxin in lanolin or hydrated lanolin was applied to the apical end of internodes. Auxin (indole-3-acetic acid) was dissolved in ethanol (0.1 M, stock solution kept in –20°C), diluted with distilled water to 100 μM and mixed with lanolin at a 1:1 weight ratio. Hydrated lanolin was prepared in a similar way except for distilled water in a place of auxin solution. The substances were renewed every three days. The basal end of an internode was immersed in tap water which was renewed every day. Control experiments consisted in treating internodes or pieces of internodes either with only auxin or only hydrated lanolin. The observations which were carried out during these experiments focused on:

1. Semi-thin sections: pieces of tissues including cambium, phloem and xylem were collected from three points on the internodes (1.5 cm from the apical and basal ends and from the middle of the internodes) and from pieces of internodes from the middle part of these pieces ("pieces of internodes" refers to internode parts obtained through their division as explained in "Results"); specimens were prepared according to the procedure described in K u r c z y ń s k a (1986).

2. Wood surface: the internodes were debarked and then immersed in an ethanol solution of phloroglucin and subsequently in HCl to stain the vessels which became visible after such a procedure; finally the wood surface of the internodes was examined under a stereomicroscope.

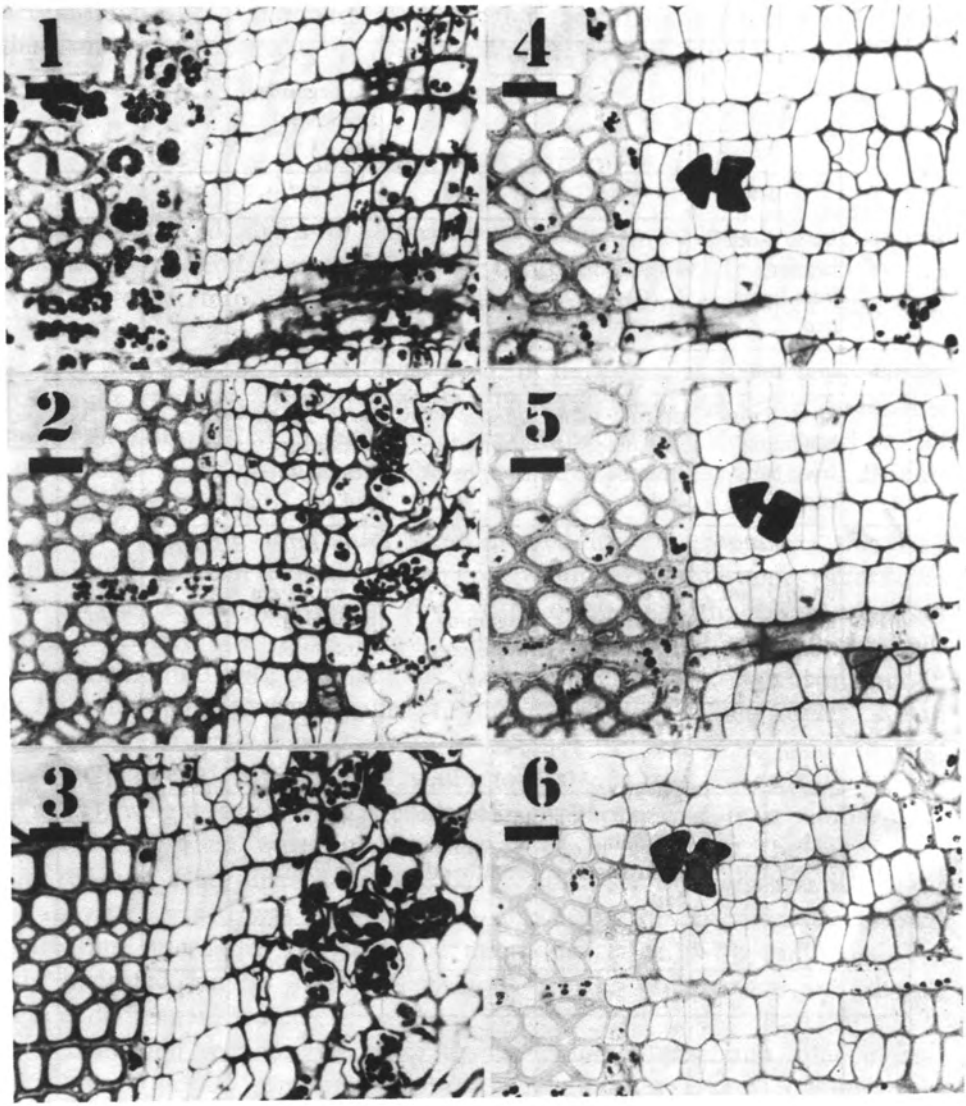
Table 1

Occurrence of tracheary elements in internodes or in pieces of internodes treated with auxin or with hydrated lanolin. Characteristics based on wood surface investigations and microscopic investigations of transversal sami-thin sections

V	1	3	5	7	9	11*
I	—	—	—	up to 4-5 cm beginning from basal end	+	+
£	3-4	4-5	4-8	6-7	9-12	9-13
II	up to 5 mm begining from basal end	+	+	+	+	
£	3-4	6-7	6-8	6-8	9-12	
III	up to 5 mm beginning from basal end	up to 2-3 cm beginning from basal end	up to 2-3 cm beginning from basal end	up to 3-5 cm beginning from basal end	+	
£	3-4	6-7	6-7	6-7	9-12	
IV	up to 5 mm beginning from basal end	up to 2-3 cm beginning from basal end	up to 2-3 cm beginning from basal end	up to 3-5 cm beginning from basal end	+	
£	3-4	6-7	6-7	6-8	8-10	
V	a —	+	+	+	+	
	b —	up to 5 mm beginning from basal end	up to 5 mm beginning from basal end	+	+	
	c —	–"–	–"–	+	+	
£	3-5	5-7	6-7	6-9	9-10	
VI	a —	+	+	+	+	
	b —	+	+	+	+	
	c —	up to 5 mm beginning from basal end	up to 1.5 cm beginning from basal end	up to 1.5 cm beginning from basal end	+	
	d —	–"–	–"–	+	+	
£	3-5	4-6	6-8	6-8	9-11	

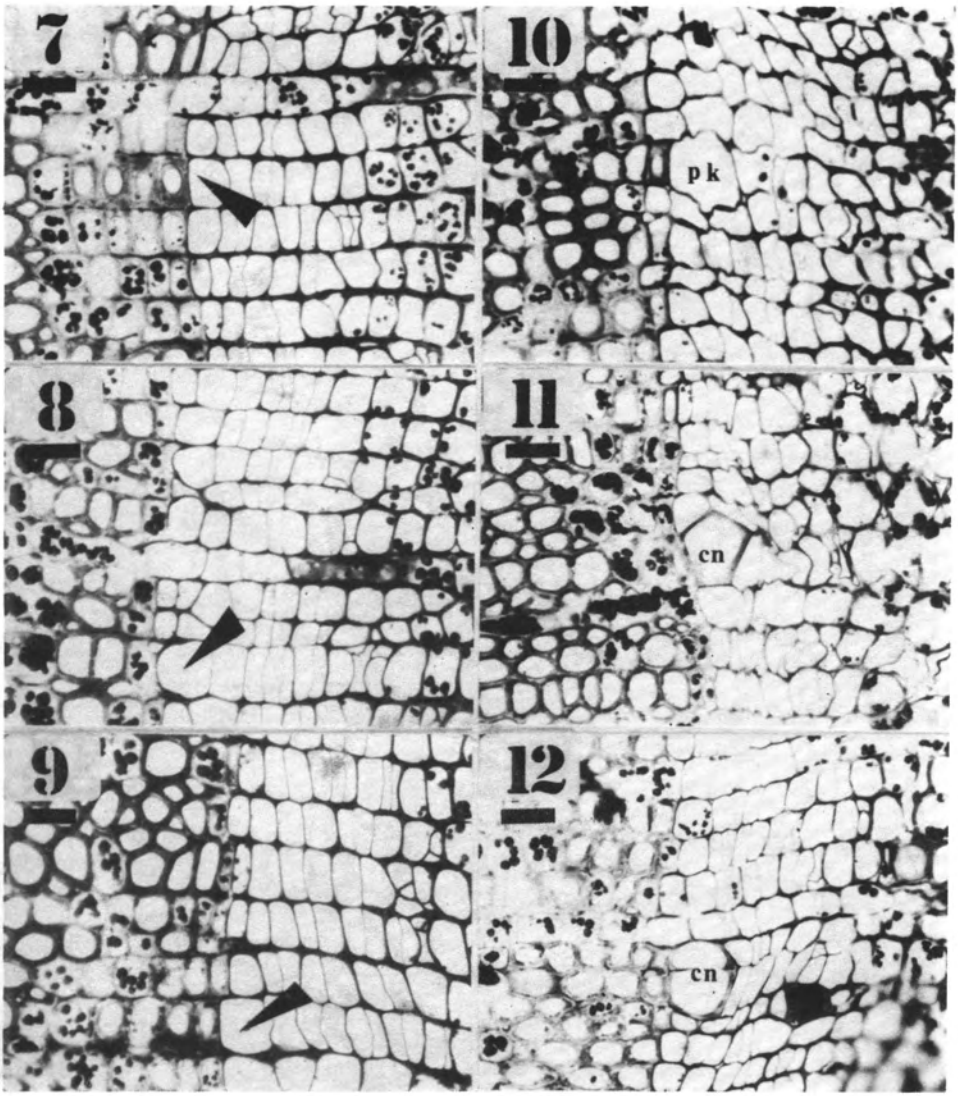
* Duration of auxin teatment (days), V — variant, "+" — presence of vessels, "-" — absence of vessels, £ — number of cambial cells, a — first (apical) piece of internode, b — second (middle) piece of inernode, c — third piece of internode, d — forth (basal) piece of internode.

PLATE I

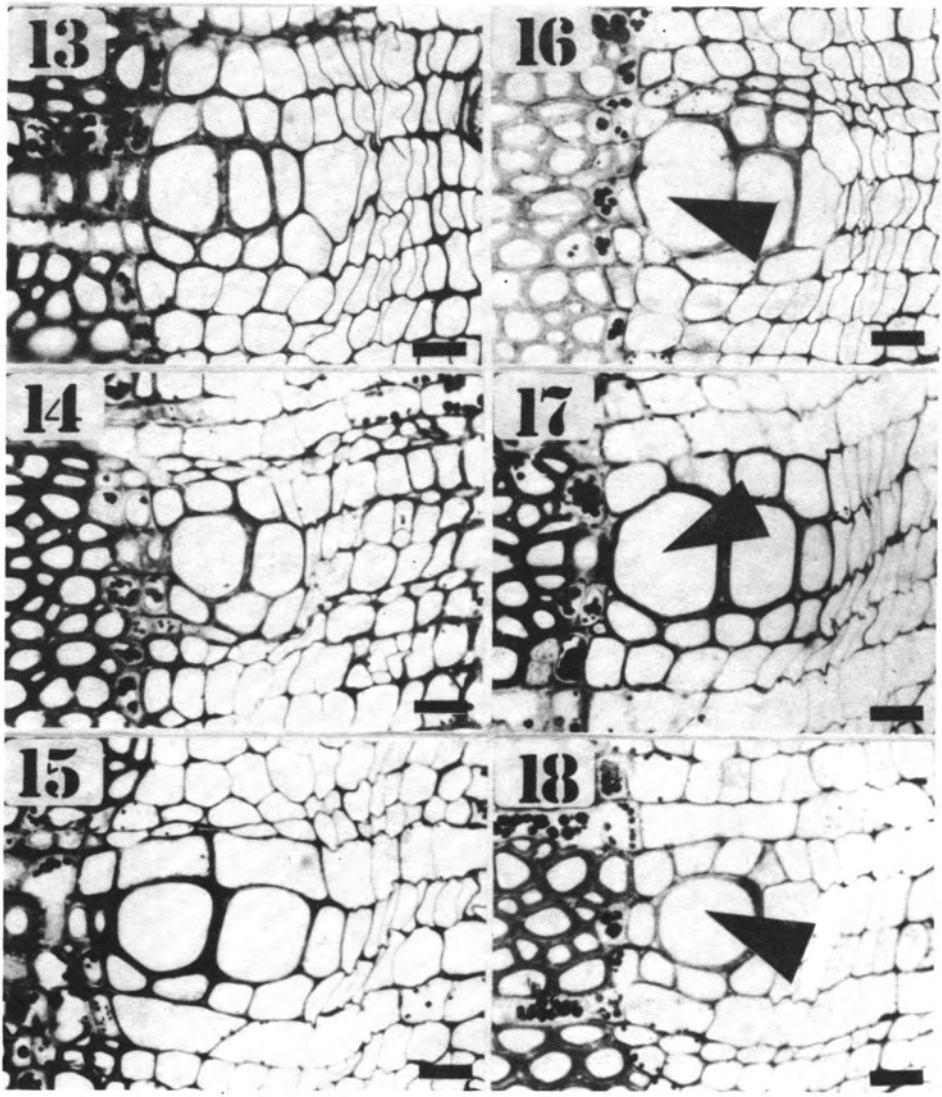


Variant I. Transversal sections made from apical (Fig. 1), middle (Fig. 2) and basal (Fig. 3) parts of internodes treated with auxin for 1 day and 3 days (Figs. 4, 5, 6). Arrows show periclinal walls. Bar = 20 μm

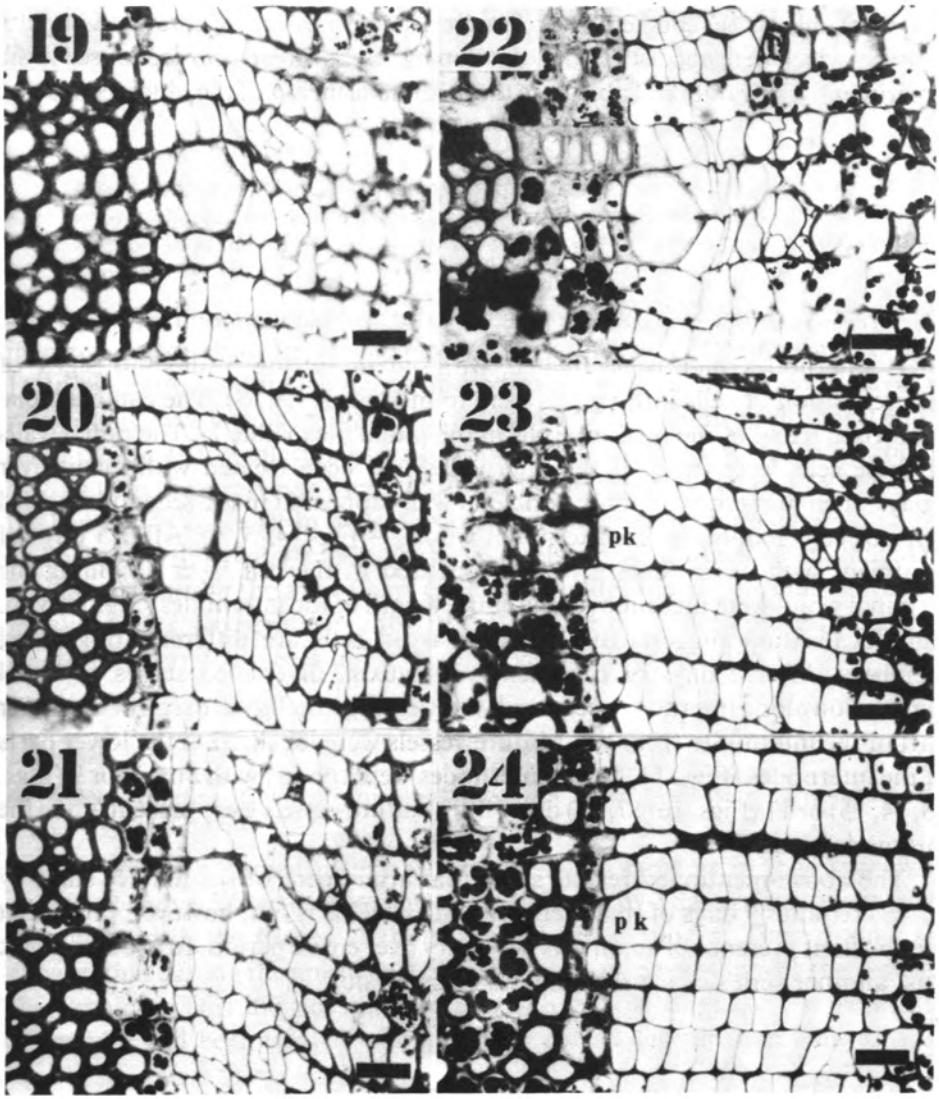
PLATE II



Variant I. Transversal sections made from apical (Fig. 7), middle (Fig. 8) and basal (Fig. 9) parts of internodes treated with auxin for 5 days (arrows show enlarged cells) and 7 days (Figs. 10, 11, 12, cn—vessel member, pk—enlarged cell). Bar = 20 μm



Variant I. Transversal sections made from apical (Fig. 13), middle (Fig. 14) and basal (Fig. 15) parts of internodes treated with auxin for 9 days and 11 days (Figs. 16, 17, 18). Arrows show vessel members. Bar = 20 μm



Transversal sections from apical (Fig. 19), middle (Fig. 20) and basal (Fig. 21) parts of internodes treated with auxin for 3 days and next treated with hydrated lanolin for 8 days (Variant II). Bar = 20 μm. Figs. 22, 23, 24 show transversal sections from middle parts of pieces of internodes which were treated with auxin for 3 days (Variant V). Bar = 20 μm

In order to determine cambial activity the number of cells between the terminal wood and phloem was counted. The cells were counted in 100 radial files (in those parts of tissues which were not disturbed by developing vessels) in each internode used in the experiment. The values in Table 1 are the arithmetic means of the obtained results.

RESULTS

VARIANT I

The internodes were treated with auxin for 1, 3, 5, 7, 9 and 11 days.

If internodes had been treated with auxin during 1 day no signs of differentiation of cells into vessels were found (Figs. 1, 2, 3). The cambial zone comprised 3 to 4 of the fusiform cells in the radial file (Table 1). The radial walls of these cells were thick. If the internodes had been treated with auxin over 3 days, then numerous dividing periclinal walls appeared in the second and third layer of cells (counting from terminal wood — Figs. 4, 5, 6). After 5 days of treatment with auxin, enlarged cells localized in the cell layer adjoining the terminal wood were present over the entire length of the internodes (Figs. 7, 8, 9). This localization suggests that these enlarged cells are differentiating vessel members. After 7 days of treatment with auxin, diversified stages of vessel maturation along internodes occurred. Only enlarged cells were seen in the upper part of the internodes (Fig. 10). Mature vessels were localized in the lower parts of the internodes (Figs. 11, 12). If internodes were treated with auxin for 9 (Figs. 13, 14, 15) or 11 (Figs. 16, 17, 18) days, fully mature vessels were present along the internodes.

The above-mentioned results show that attainment of the mature stage by vessels requires 9 days of treatment with auxin. This is not, however, proof that the constant presence of auxin was necessary over entire period. In order to check this, another series of experiments was carried out.

VARIANT II

Internodes were treated with auxin for 1, 3, 5, 7 and 9 days, then auxin and 2-3 mm of internodes from the apical end were removed. Hydrated lanolin was then applied to this "new" surface for such a period that the total duration of the experiment was 11 days (e.g. 3 days auxin + 8 days hydrated lanolin).

If internodes had been treated with auxin for only 1 day of the 11-day experiment, the cambial activity and vessel differentiation remained the same as described in variant I (Table 1). If internodes had been treated with auxin for 3, 5, 7 or 9 days of the 11-day experiment then vessels were differentiated along the whole length of the internodes (Figs. 19, 20, 21, Table 1). These results indicated that the 3-day treatment with auxin sufficed for vessel differentiation and

maturation. However, since it may have been possible that some auxin remained in the apical tissues, another variant of the experiment was performed to check this.

VARIANT III

After 1, 3, 5, 7 and 9 days of treatment with auxin, 1/3 of internode length from its apical end was cut off, then the remaining part of the inernode was treated with hydrated lanolin for a period so as the total time of culture was 11 days (e.g. 3 days auxin + 8 days hydrated lanolin).

If the internodes had been treated for 1 day with auxin before being cut off, then no new vessels were found in pieces originating from these internodes (Table 1). In pieces originating from internodes treated with auxin for 3 or 5 days, the new vessels were localized in the region between 2 to 3 cm from the basal end of these pieces. If the internodes had been treated with auxin for 7 days before cutting then the new vessels were localized in the region between 3 to 5 cm from the basal end (Table 1). In the pieces of internodes treated for 9 days with auxin and next for 2 days with lanolin, the new vessels were cut, which means that at the moment of cutting the apical part of the internodes the vessels must have been mature. Control experiments showed that neither the cutting procedure nor the length of internode pieces influenced the vessel differentiation.

VARIANT IV

These experiments differed from those in variant III only by having 1/2 of the length of internodes cut off.

The results obtained in this variant were the same as in the experiments described above. These results showed that at most by 3 days of treatment, auxin accumulated in the tissues at the basal end of internodes. These results also showed that cutting the apical part of internode was the reason why the vessels in the remaining part did not matured along the whole length of the piece if the internodes had been treated with auxin for period shorter than 9 days.

VARIANT V

The internodes were treated for 1, 3, 5, 7, and 9 days with auxin, then auxin was removed and the internodes were divided into 3 equal pieces. Each piece was then treated with hydrated lanolin for a period so that the total time of the experiment was 11 days (e.g. 3 days auxin + 8 days hydrated lanolin).

If the internodes had been treated with auxin for only 1 day the results were the same as in the variants described above. If the internodes had been treated for 3 or 5 days with auxin before being divided, vessels appeared only in the apical pieces of internodes (Table 1, Fig. 22). Only enlarged cells were seen in the middle

and basal pieces (Figs. 23, 24). If the internodes had been treated with auxin for 7 or 9 days before dividing, the vessels were differentiated in all the pieces of internodes (Table 1). The results from this variant confirmed the earlier conclusion that auxin accumulated in the tissues in the vicinity of the apical and basal ends.

VARIANT VI

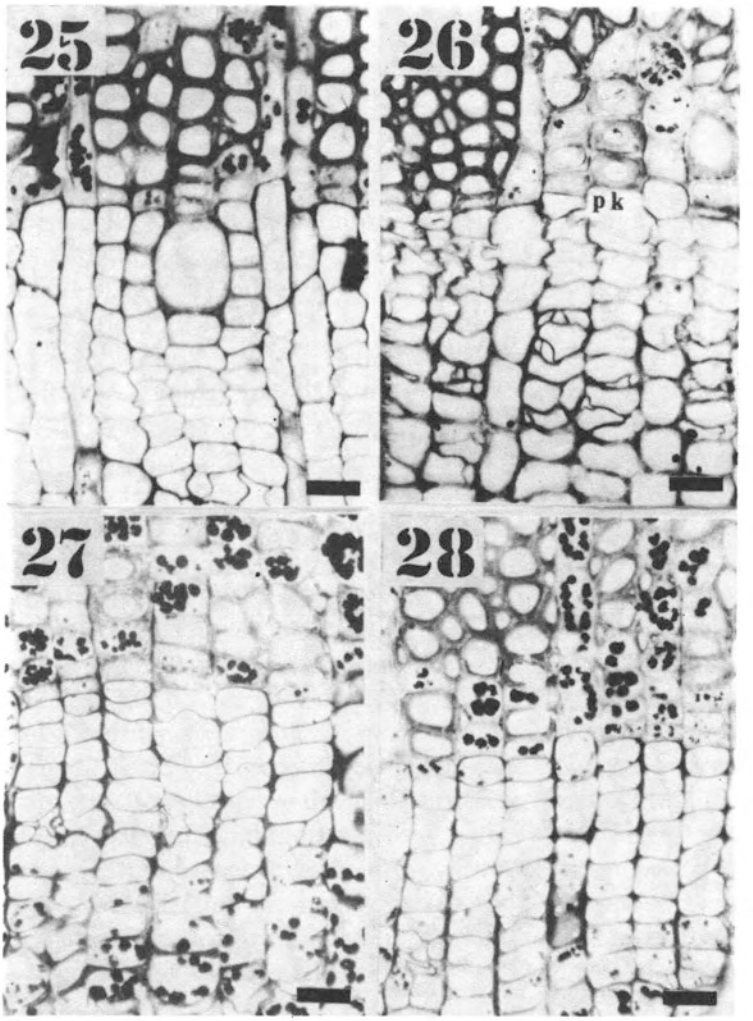
These experiments were similar to those in variant V but with only one difference, namely, internodes were cut into 4 equal pieces.

If the internodes had been treated with auxin for 3 days and next divided into 4 pieces, vessels differentiated only in the first (apical) piece (Figs. 25, 26, 27, 28). If internodes were divided after 5 days of treatment with auxin then differentiated vessels were found in the first (Fig. 29), second (Fig. 30) and forth (Fig. 32) pieces of internodes. In the third piece only enlarged cells were seen (Fig. 31). If internodes had been treated with auxin for 7 or 9 days, then the vessels were differentiated in all of the pieces of these internodes (Table 1).

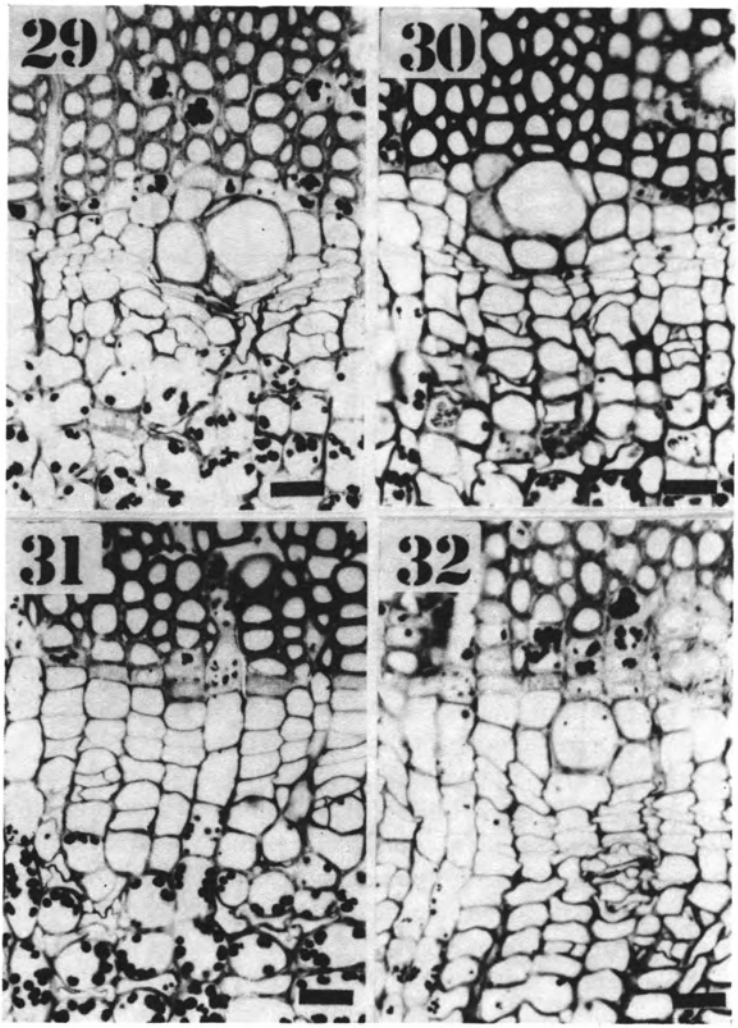
Hand-cut sections through the cambium, phloem and xylem were made in order to determine the anatomical characteristics of internodes at the moment of: changing the auxin into hydrated lanolin, cutting off the apical part of internodes and dividing internodes into pieces. Microscopical analysis of these sections showed that at those moments if the internodes had been treated with auxin for 5 days there only enlarged cells were in the cambial zone.

Basing on the results described above we can say that 1 day of treatment with auxin had no influence on cambial activity and vessel differentiation. Auxin had to act for at least 3 days to cause cell divisions in the cambial zone. After 5 days of treatment with auxin the first signs of vessel differentiation were visible (enlarged cells). The enlarged cells appeared simultaneously along internodes. Differences in vessel maturation were visible after 7 days of treatment with auxin. Nine days of treatment with auxin produced fully mature vessels. Maturation of the vessels which differentiated at the basal end of the internodes was faster than in the other parts of these internodes. The number of cells between terminal wood and phloem increased together with lengthening of the duration of auxin treatment. The obtained results showed that continuous treatment with auxin not only had a qualitative but also a quantitative influence on the process of vessel differentiation and cambial activity.

As mentioned in "Material and Methods", two kinds of control experiments were carried out. In the first, all of the internodes or pieces of internodes were treated with only auxin. In that case, vessels were differentiated in all of the internodes. In the second control experiment, internodes or pieces of internodes were treated only with hydrated lanolin. In that case new tracheary elements differentiated only in the 5 mm area at the basal ends of the internodes.



Variant IV. Transversal sections from middle parts of pieces originating from an internode which was treated with auxin for 3 days (Figs. 25, 26, 27, 28). Bar = 20 μm



Variant VI. Transversal sections from middle parts of pieces originating from an internode treated with auxin for 5 days (Figs. 29, 30, 31, 32). Bar = 20 μm

DISCUSSION

It can be assumed based on the obtained results that in the case of ash internodes, auxin was necessary for vessel differentiation. This is consistent with the results of investigations on other objects (K a l d e w e y 1984). The obtained results also permit us to conclude that the role of auxin in the process of vessel differentiation is related to its property of basipetal transport in the cambial region. Namely, taking into consideration the length of the internodes used in the experiments and the velocity of polar transport of auxin (6 mm/h — K u r c z y ń s k a and M i c h a l c z u k 1989), the time lapse to the first signs of vessel differentiation is long enough to consider molecular transport of auxin to be a factor responsible for vessel differentiation. Experiments carried out on ash internodes showed application of auxin at the basal end of internodes did not cause vessel differentiation (Kurczyńska — unpublished). This confirms the conclusion drawn above concerning the relationship between the polar transport of auxin and vessel differentiation.

The finding of the relationship between the polar transport of auxin and vessel differentiation can be interpreted in two ways. Namely, the role of the polar transport of auxin in differentiation of vessels can depend either on the flow of auxin through the cells which differentiate into the vessels (S a c h s 1981) or on supplying sufficient amounts of auxin into these cells (J a c o b s and M o r r o w 1957). In the first case the receptor of the information carried by auxin could be an analog of a counter measuring the number of conveyed auxin molecules. In the second case it could be a receptor recognizing the auxin concentration inside the cell. From results presented in this paper it is seen that the first signs of vessel differentiation appeared simultaneously along the whole length of the internodes. However, from earlier investigations with labelled auxin carried out on ash internodes, we know that the auxin concentration varies at different levels of internodes: the auxin concentration is very high in the vicinity of the apical and basal ends as compared with the remaining part of the internodes (K u r c z y ń s k a and M i c h a l c z u k 1989). Thus, the appearance of enlarged cells simultaneously along the internodes with such high differences in auxin concentration showed that at the first stage of vessel differentiation, the polar flow of auxin plays the main role. This is consistent with Sachs' hypothesis (1981) according to which vessel differentiation depends on the polar flow of auxin through cells.

The simultaneity discussed above refers to the enlargement of cells differentiating into vessels but does not refer to the maturation of these vessels. The earliest matured vessels were at the basal end of internodes. From the paper mentioned above (K u r c z y ń s k a and M i c h a l c z u k 1989) it is known that the auxin concentration is particularly high at the basal end. Is it possible then to imply any connection between the earlier vessel maturation at the basal ends of internode and the auxin concentration? Because at the apical ends of internodes

the auxin concentration is high as well (Kurczyńska and Michalczuk 1989) we could expect that in this part of internodes also, vessels could mature earlier than the vessels in the middle parts of the internodes. But this is not true, as shown by the results presented in this paper. The auxin concentration at the apical ends is high but it is half that at the basal end of the internodes (Kurczyńska and Michalczuk 1989) and probably not sufficiently high to cause earlier vessel maturation. Thus, the earlier vessel maturation at the basal end of the internodes could be associated with auxin concentration but on the assumption that auxin concentration should attain a sufficiently high level.

The results presented in this paper allow us to assume that vessel differentiation depends on auxin flow and only the last stage of vessel differentiation, namely, vessel maturation depends on auxin concentration. Such an interpretation of the results could reconcile Sachs' hypothesis with the results of xylogenesis induction in tissue cultures, from which we know that suitable auxin concentration is a sufficient factor for induction of tracheary elements, i.e. cells with lignified, pit walls (Fukuda and Komamine 1980).

The results presented in this paper also show that auxin is necessary during most if not the entire period of cell differentiation into vessels. Namely, cells which increased in size during auxin action (this means that these cells were determined into vessels) did not maturate after auxin removal. This means that auxin is necessary not only for determination but also for maturation of cells into vessels, which is consistent with results obtained during the experiments with herbaceous plants (Sachs 1974).

Acknowledgment

The author is deeply grateful to Professor Zygmunt Hejnowicz for his aid, valuable advice and critical comments during the course of this work.

REFERENCES

Fukuda H., Komamine A., 1980. Direct evidence for cytodifferentiation to tracheary elements without intervening mitosis in a culture of single cells isolated from the mesophyll of *Zinnia elegans*. Plant Pysiol. 65: 61-64.

Jacobs W.P., 1952. The role of auxin in differentiation of xylem around a wound. Amer. J. Bot. 39: 301-309.

Jacobs W.P., Morrow I.B., 1957. A quantitative study of xylem development in the vegetative shoot apex of *Coleus*. Amer. J. Bot. 44: 823-842.

Kaldewey H., 1984. Transport and other modes of movement of hormones (mainly auxins). Encyclopedia of Plant Physiol. New Series, Vol. 10, Hormonal Regulation of Development II. Springer-Verlag, Berlin-Heidelberg.

Kurczyńska E., 1986. Terminal vessel and early vessel arrangement in internodes of *Fraxinus excelsior*. Acta Soc. Bot. Pol. 55: 3-10.

Kurczyńska E.U., Michalczuk L., 1989. The distribution of radioactivity along isolated segments of ash stem treated with labelled auxin. Acta Physiol. Plant. 2 (11).

Roberts L.W., 1976, Cytodifferentiation in plant xylogenesis as a model system. Cambridge University Press.
Sachs T. 1974, The induction of vessel differentiation by auxin. In: Proceedings 8th International Conference on Plant Growth Substances, Hirokawa — Tokyo.
Sachs T., 1981. The control of the patterned differentiation of vascular tissues. Adv. Bot. Res. 9: 151-262.
Wetmore R.H., Sorokin S., 1955. On the differentiation of xylem. J. Arnold Arbor. 36: 305-324.

Auksyna a różnicowanie naczyń w izolowanych międzywęźlach jesionu (Fraxinus excelsior L.)

Streszczenie

Międzywęźla jesionu pobierano z warunków naturalnych w okresie spoczynku zimowego. W celu pobudzenia aktywności podziałowej kambium i różnicowania naczyń podawano auksynę. Czas podawania auksyny był różny w różnych grupach międzywęźli. Stwierdzono, że auksyna konieczna jest nie tylko do indukcji komórek na naczynia, ale również w trakcie ich dojrzewania. Uzyskane wyniki przedyskutowano w oparciu o koncepcję Sachsa.